Advances in Genomics: Applications to Banana Breeding

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Abstract

Like many vegetatively propagated crops, most cultivated bananas are highly heterozygous polyploids of mono or interspecific constitution. During human selection for edibility over thousands of years, *Musa* cultivars developed a particularly high level of male and female gamete sterility associated with a low fertilizing potential making genetic recombination through meiosis and hybridization rare events during domestication. Recent evolutionary studies in *Musa* suggest that only a few hybridization events occurred from the wild seedy primitive ancestors to the most evolved present day cultivars. Unlike other crops, in such a context where the species complex is nearly fixed, banana breeding relies on strategies where an extremely limited number of crosses are possible. Several genomic tools are now being used to decipher the complexity of the *Musa* genepool. Molecular markers can distinguish between genotypes and have provided a clear understanding of banana domestication suggesting only a few hybridization events from the wild seedy primitive to the present day varieties. This allows us to build breeding schemes mimicking the sequence of crossings and selections that occurred over several millennia. The *Musa* genome can now be explored for desirable genes and subsequent marker-assisted selection. For banana improvement, this approach is particularly important in a biological context of gamete sterility (and difficulties to get large quantities of seeds) for which recombination and implementation of introgression strategies remain the greatest challenge.

INTRODUCTION

A Giant with Feet of Clay

Despite recurrent announcements predicting its future decline, global banana production since the sixties never ceased progressing to reach today about 145 million tons per year. Around 85% of the global production, mostly produced in non-intensive cropping systems (backyard gardens, small plots, small farms), are dedicated either to self consumption, or local and regional markets. The remaining 15% produced in highly intensive conditions by the agribusiness industry are exported on international markets towards developed countries.

Nevertheless, despite the lasting increases in global banana production, associated agrosystems are fragile, and diseases are spreading. Indeed nearly 95% of global production relies on just a few genetic combinations, involving a very limited number of meiotic events (from 7 to 14 at the most) with one single recombining event only (Cavendish) representing half of the total production, export and local markets taken together.

There are good reasons that can explain the supremacy of Cavendish cultivars: they combine good fruit quality, good post-harvest behavior, high agronomic performance and a high adaptability to contrasting environments and agro-ecological zones.

Such a critical lack of cultivated genetic diversity for a crop of major importance as staple food for millions of people is an exception. There is no equivalent in the rest of the plant kingdom, even in vegetatively propagated crops like potatoes or strawberries. These crops are also polyploids but have not been selected for gamete sterility as has been the case for cultivated bananas.

During banana domestication, man selected for sterility. Consequently, in the absence of genetic recombination, the present banana complex of cultivated cultivars is nearly completely frozen, with no further evolution at the polyploidy level, except the occurrence of somatic mutations or bud-sporting due to strict vegetative propagation (i.e. cloning) over long periods leading to amplification of phenotypic diversity.

These genetically restricted and inflexible clones are particularly susceptible to diseases, pests, and current ecological changes. Banana cultivation is thus an amazingly favorable configuration for pests and disease proliferation where pathogens, unlike banana, are highly adaptive. The ongoing emergence of new resistant strains of *Mycosphaerella fijiensis* to fungicides is a prime example.

Finally, almost all the cultivars are susceptible to one or various pathogens and if one cultivar is infected, its somaclones will eventually be infected too. In the absence of control by crop husbandry or pesticides, pests and diseases play a significant role in reducing banana yields to far below the potential. For example, yields of Plantain and East African highland banana in Africa are 2 and 6 t/ha, respectively, where the potential is 20 to 35-45 t/ha, respectively.

There is thus an urgent need to broaden the genetic base of cultivated cultivars by the release of new cultivars, resistant to pest and diseases, taking advantage of the richness of the still underexploited banana genetic diversity, including the crop's wild relatives.

BETTER UNDERSTANDING OF BANANA EVOLUTION FOR BETTER BREEDING STRATEGIES

It is commonly known that the two diploid seedy species *Musa acuminata* and *Musa balbisiana* are the ancestors of the several hundred diploid and polyploid edible cultivars, originating from intra- and interspecific hybridizations (Heslop-Harrison and Schwarzacher, 2007). This general rule admits some exceptions such as contributions from *Musa textilis* to Fei's and from *Musa schizocarpa* to some diploid cooking types that originate from Papua New Guinea (Sharrock, 2000; Carreel et al., 2002).

But, for the breeders, if these results are significant, they nevertheless remain insufficient to explain the resounding status of some monocrops such as Cavendish (worldwide), Plantains (in West and Central Africa), East African highland banana or Prata in Brazil. Emeritus plant breeding Professor Yvette Dattée, president of the *Société Nationale d'Horticulture de France*, visiting the CIRAD banana improvement program in Guadeloupe in 1995, said: "the day we will be able to understand why a single genotype [Cavendish] accounts for nearly half of world production, is the day we will be able to run a good banana improvement program".

We have tried to give part of the answer through a better knowledge of the detailed genomic constitution of the present day cultivars, through the understanding of the role of their genetic/genomic background in explaining their extension at the global scale (Perrier et al., 2009, 2011).

Of course, banana breeding does not aim solely at genetic improvement for Cavendish, and the choice of genetic resources for use in banana breeding must fit with

the objectives of the program, i.e. the landraces one aims to improve. Then, it is obvious that breeders should not mix any genotype for the synthesis of new cultivars to substitute specific traditional landraces. Indeed, dessert-associated genetic resources are not convenient for cooking cultivars and the reverse is also true, although not completely as we shall see later.

In the first place, the tools offered by genomics have allowed finding answers to the questions raised about the evolution of bananas, i.e. how current cultivars have reached us. Unlike other crops such as cereals or maize, banana evolution must be considered as a discontinuous process of successive thresholds (Fig. 1).

From Seedy to Edible Diploids

Nuclear molecular markers differentiate *M. acuminata* wild diploids into four distinct genetic clusters, which fit with four of the subspecies defined using morphological characters: (1) ssp. *banksii* cluster from New Guinea, (2) ssp. *malaccensis* (Ridl.) Simmonds cluster from the Malayan Peninsula, (3) ssps. *burmanica* Simmonds, *burmanicoides* De Langhe and *siamea* Simmonds from northeast India, Myanmar, southern China and Thailand, and (4) ssps. *zebrina* (Van Houtte) / *microcarpa* cluster from Java.

Diverse wild *M. acuminata* subspecies have probably been subject to progressive domestication in their native regions, so called cultiwilds (De Langhe, 2009). The emergence of protoagriculture initiated a long-term selection process with progressive evolution from wilds to cultiwilds and then to protocultivars defined as intermediate edible forms being no longer cultiwilds but not yet basic cultivars. It is a matter of fact that useful parts of banana plants were potentially exploited over millennia for food, fodder, medicine, fiber, domestic uses, or construction materials.

This process might have occurred in parallel and independently in the various areas of *M. acuminata* subspecies. Pulp development which is under genetic control may have been positively selected during the cultiwild phase, giving birth to protocultivars. Evidence of this selection might be a wild Samoan *banksii* that shows a partial pulp development although it is perfectly fertile (Simmonds, 1962). The same case has been noticed in the wild *malaccensis* ‘Pisang Serun’ (Rosales et al., 1999).

A second key element relevant to the domestication history provided by genomics is that edible diploids derive from hybridizations between different *M. acuminata* subspecies. It is likely that the protocultivated forms were spread by humans as they explored surrounding territories. Indeed, molecular markers showed that AA edible diploids never overlap with seeded subgroups and are always very heterozygous, suggesting being hybrids between genetically distant forms. The allelic contributions of the main subspecies to these AA cultivars often show the contribution of at least two subspecies. In addition, all these AA edible diploids present structural heterozygosity reflecting chromosomal rearrangements between parental subspecies of *M. acuminata*. Combined with parthenocarpy, this structural and genic heterozygosity promoted by human migrations between islands of Southeast Asia and western Melanesia has contributed to gamete sterility and edibility of seedless fruits.

Some interspecific AB cultivars (cvs) have also occurred particularly in India where presumably AA edible diploids pollinated seedy *balbisiana* wild types.

From Edible Diploids to Triploid Cultivars

A further consequence of hybridity in edible AAcvs and ABcvs was erratic meiosis, thereby occasionally producing unreduced gametes (Simmonds, 1962). A direct consequence of fusion of diploid gametes with haploid gametes was the emergence of triploid genotypes AAA and AAB or ABB by interspecific hybridizations, leading to the diversity of the current cultivated triploids, under human selection via vegetative propagation. Triploidization occurred independently in various contact areas likely between edible diploids as donor of 2n gametes and cultiwilds or protocultivars, but still gamete fertile. It started probably early after the AAcv emergence and is certainly still ongoing, as demonstrated by the recent origin inferred for the genome of several AAAcvs in New Guinea (Perrier et al., 2009).

Molecular phylogenetic analyses have identified the diploid clusters contributing to modern triploid cultivars. These clusters are groups of cultivars genetically close together either by sexuality or vegetative propagation. *M. acuminata* ssp. *banksii* derivative cultivars played a major role in the emergence of several important diploid and triploid cultivar groups that are now widely spread (Hippolyte et al., 2012). For example, *M. acuminata* ssp. *zebrina*- and ssp. *banksii*-derivative AAcvs contributed to AAA East African Highland bananas. Likewise, the A genome of the AAB cooking bananas Plantains, Maoli and Iholena of West Africa and the Pacific is of pure *banksii* origin.

In dessert bananas, the 2n gamete at the origin of Cavendish and Gros Michel cultivars is genetically close to 'Akondro Mainty', an AAcv of the "Mlali" cluster, while the n gamete was given by accessions belonging to a cluster of diploid cultivars called "Khai" (Fig. 2), (Raboin et al., 2005; Perrier et al., 2011). At this point, it is interesting to focus on the "Mlali" cluster, which includes a set of cultivars that are only found in East Africa under different names ('Mshale', 'Muraru', 'Ndyali', etc.) and the neighboring islands: Madagascar, Comoros, Zanzibar and Pemba. This is clearly an ancient group coming from a cross between *banksii* and *zebrina/microcarpa* genomes somewhere between Java, Borneo and New Guinea. Surprisingly, Mlali cultivars are apparently no longer recorded in this area.

So, Cavendish and Gros Michel cultivars can be considered as three-way cross hybrids resulting from the cross of an F1 hybrid *banksii* x *zebrina/microcarpa* somewhere between New Guinea and Indonesia and a *malaccensis* genome from the Malayan Peninsula. By their specific genomic constitution, these cultivars present the highest level of heterozygosity that can be found in triploid AAA bananas. This could explain the remarkable plasticity of these cultivars and their high agronomic performance giving thus a part of the explanation to their dissemination throughout the world. It must be noticed here that, more than triploidy itself, the fruit qualities of Cavendish or Gros Michel have to be related to their Mlali origin, where some clones show very similar fruits.

In Sweet acid bananas (AAB group), the results of genomic research suggest that Silk cultivars originate from a cross between an AA *malaccensis* derivative diploid (belonging to the "Khai" cluster) and a *balbisiana* wild relative (close to the current Indian 'Lal Velchi' accession). In another way, Pome cultivars (like 'Prata', currently cultivated in intensive cropping systems in Brazil to supply the domestic market) originate from a cross involving an AAcv of the "Mlali" cluster and a *balbisiana* close to the same 'Lal Velchi'. Like Cavendish, Pome cultivars are thus highly heterozygous containing three different genomes, a *balbisiana* genome and two distinct *acuminata* genomes (*banksii* and *zebrina*).

A major conclusion drawn from genomic studies regarding banana evolution is that very few recombining events occurred from the primitive forms to the most advanced

triploid cultivars: one cross between diploids to raise to the triploid level, maybe two to five crosses to reach edibility at the diploid level in a context of decreasing gametic fertility and probably no more than three to ten crosses from wilds to protocultivars. Back-crosses may have occurred between some tetraploids and diploids (De Langhe et al., 2010) but they have certainly been rare events.

These scenarios are based on data provided from "old" molecular tools (RFLP, SSR, DArT). It is likely that implementation of latest technologies based on Next-Generation sequencing will refine this vision of banana evolution. Such technologies include resequencing, genotyping by sequencing and any other method aiming to saturate and locate genetic information on the genome. They may contribute, for example, to highlight the mosaic structure of the chromosomes in edible diploids thereby clarifying recombination events at the diploid level.

BREEDING BY RECONSTRUCTION

Triploid hybrids are thought to be the best objective for banana breeding, as triploidy gives a selective advantage over the other ploidy levels. Diploids are not suitable as final breeding products: they usually are less productive and less vigorous than triploids, with smaller bunches and sometimes containing seeds in fruits. However, some commercial trades of significant importance rely on diploids like 'Kunnan' (ABcv) in India or 'Pisang Mas' / 'Sucrier' (AAcv) in Colombia but for high value niche markets far from mass consumption. Tetraploidy does not seem appropriate too. Actually, bunch and fruits may be equivalent to triploids but postharvest qualities never meet the requirements of the regional and international trades as fresh fruits and fruits are poor substitutes for processing. This might be due to the higher water content in tetraploid tissues when compared to diploid and triploid cultivars.

From a banana breeder's perspective, rather than an obstacle, triploidy should be thought as a fantastic opportunity to combine three or more distinct genetic pools in a single genome to maximize heterozygosity and to the benefit from all the favorable consequences arising thereof (Gallais, 2009).

Several years ago, we developed at CIRAD an original breeding strategy relying on the use of the diploid genetic stocks to create triploid hybrids, called "reconstructive breeding", in opposition to "evolutionary breeding" schemes relying primarily on triploid per diploid crosses (Tenkouano et al., 2011). Recent advances in the understanding of the evolutionary pathways of the banana complex promoted by genomic studies reinforced this approach, by determining the most likely diploid ancestors of present day triploid cultivars and allowing more relevant choices of parental combinations.

However, phylogeny is not the only pillar of our breeding strategy. It is also based on a search of the best specific combining abilities between two diploids of which one is the donor of diplo-gametes. Based on complementarities, this approach aims to associate the favorable traits brought by both parents and to maximize the heterozygosity in the triploid progenies (Sanford, 1983). But, since the production of 2n gametes is uncontrolled and fairly rare in diploid clones (Dodds, 1943), a more regular production of 2n gametes is expected from chromosome doubling (Bakry and Horry, 1994; Bakry et al., 2007).

The ability to set progeny in banana is strongly linked to gamete fertility that is highly variable from clone to clone and chromosome doubling is not an insurance to restore fertility. The gamete fertility of the doubled AA diploids is rather unpredictable: some clones are slightly fertile at both diploid and tetraploid levels, while others are completely sterile at the tetraploid level. Conversely, all interspecific AB clones are sterile

at the diploid level, but showed to be systematically male and female fertile at the tetraploid level (pers. obs.), indicating that gamete sterility in AB clones is probably due to incomplete chromosome pairing at meiosis (Jeridi et al., 2012). This situation is particularly favorable for breeding interspecific AAB and ABB hybrids.

Breeding for Cavendish-like Bananas

Work has focused on the use of 'Mlali' AAcv accessions we introduced from the Comoros islands. We selected a set of these based on agronomic performance and, after chromosome doubling, they were tested in crosses with AA accessions of the "Khai" cluster. Despite the low fertility of most of the doubled-diploids (Goigoux et al., 2013), some triploid progenies were obtained showing highly variable performances. Among the progenies, one individual was obtained, looking very similar to 'Lacatan', the giant form of Cavendish, both in terms of plant stature and in fruit quality, demonstrating thus the proof of concept of this novel breeding approach (Fig. 3). Its susceptibility to Sigatoka disease came as no surprise, as both Mlali and Khai parents are susceptible.

Natural diploid AAcv progenitors belonging to the "Khai" cluster (*malaccensis* background) and combining gamete fertility, resistance to diseases and favorable agronomic features are rare. Therefore there are needs for pre-breeding within the "Khai" cluster aiming to develop fertile improved progenitors containing various sources of disease resistant genes derived from crosses between edible diploid clones and wild relatives. It is likely that the exploitation of *Musa* genetic maps, the development of high density molecular markers, and the capacity in the near future to identify various allelic forms of numerous genes will help in the implementation of these new pre-breeding activities, close to a "Breeding by design" approach.

Breeding for Sweet-acid Bananas

Sweet-acid bananas (AAB Silk and Pome) are highly susceptible to Fusarium wilt and Sigatoka diseases. Breeding programs aim at the development of new disease resistant sweet-acid banana cultivars that retain the organoleptic and productivity of the traditional landraces. Common breeding strategies for these groups consisted so far in the selection of tetraploid hybrids, directly issued from crosses between AAB sweet-acid cultivars pollinated with AA clones carrying disease-resistance genes. However, this crossing pathway is hampered by low gamete fertility and the rare occurrence of desired 2n gametes on the AAB female side. On our side, we tested, within a "reconstructive approach", the synthesis of triploid hybrids directly from the 'Kunnan' landrace (ABcv), chosen because of its high usage value in India. Its genomic constitution suggests that it would come from a cross between a *malaccensis*-derived edible diploid and a *balbisiana* wild relative.

As already mentioned, natural AB clones are sterile but their AABB neo-allotetraploid counterparts obtained through colchicine treatment are fertile. Both as male and female parent, the gamete fertility of the tetraploid 'Kunnan' was thus used in crosses with AA and BB accessions to generate directly triploid hybrids (Jenny et al., 2013). In crosses with wild and edible disease resistant *malaccensis* derivatives, we obtained several AAB hybrids ranging from Silk-like to Mysore-like (another AAB important sweet-acid banana) cultivars, and resistant to Fusarium wilt and Sigatoka leaf streak diseases (Fig. 4). On the other hand, in crosses with *balbisiana* accessions, several ABB hybrids developed were very similar to 'Pisang Awak' clones.

These are preliminary results. But, by its capacity to gain advantage from any bi-parental combination (including unimproved wild relatives and/or any combined source

of resistance), this new means of creating interspecific triploids seems to offer limitless potential.

In the near future, it is likely that advances in genomics will support these new strategies, moving marker-assisted selection forward and facilitating recurrent improvement of the diploid seedy parents for subsequent introgressions in the final triploid product.

Breeding for Cooking Bananas

Breeding strategies currently implemented for cooking bananas are mostly based on crosses between Plantains (AAB) as female parents and improved disease-resistant AA diploids as the male parent. The selected disease-resistant primary tetraploids (AAAB) can be either released as new improved cultivars or subsequently mated with other improved diploid selections to obtain secondary triploids.

Some outstanding hybrids were obtained by this strategy. FHIA-21, a cooking tetraploid hybrid released by the *Fundación Hondureña de Investigación Agrícola* (FHIA, Honduras), is now being cultivated for local markets in some countries in West Africa, in Central and South America and in the Caribbean. These hybrids are resistant to Black Leaf Streak disease, with, for some of them, better agronomic characteristics than the corresponding triploid maternal cultivar (Vuylsteke et al., 1993). Unfortunately, the release of these tetraploid hybrids has been hampered by the presence of endogenous integrated sequences of the Banana streak virus (eBSV) in the Plantain genome. Activated during hybridization, these sequences release infectious viral particles in their progenies. However, it has been shown that these viral sequences behave as pseudo-genes and can in some cases segregate as a heterozygous locus (Gayral et al., 2008). Thanks to redistributions and recombination between A and B chromosomes during meiosis, triploid hybrids free from infectious eBSV were obtained from AAAB x AA crosses. Marker-assisted selection (PCR, southern blot) is now exploited to release new cooking hybrids free from any infectious eBSV. A plantain-like hybrid obtained at *the Centre Africain de Recherches sur Bananiers et Plantains* (CARBAP) in 2012, 'K74', is a first and nice illustration of this new strategy (pers. observ.).

Alternatively, the high potential of the neo-allotetraploid pathway for sweet-acid banana improvement (see above) offers a wider range of possibilities in breeding for cooking bananas. In a first step, edible diploid hybrids are created from crosses between *M. balbisiana* as the female parent and diploid "cooking type" cultivars from a *banksii* origin. Following chromosome doubling, the BA-BA neo-allotetraploids are crossed with other AA cooking genotypes in a second step. Then, it becomes possible to combine maximization of heterozygosity, incorporation of new sources of resistance and non-infectious eBSV patterns in the selected triploid hybrids. First preliminary results are promising, but, unfortunately, the BA diploid hybrids obtained to date showed infections with BSV particles resulting likely from activation of eBSV brought by the *balbisiana* parents. Consequently, we develop a pre-breeding approach seeking a *M. balbisiana* free of infectious eBSV concurrently with the preservation of a high fertility and favorable agronomic features. Designed molecular markers such as immunocapture–PCR and southern-blot have been developed and are routinely used at CIRAD to support such a strategy (Umber et al., 2011; see also Pichaut et al.'s contribution, this symposium).

CONCLUSION

In the recent decades, several genomic tools have been successfully used to decipher the complexity of the *Musa* genepool. Molecular markers have not only proven

to be effective to distinguish between genotypes, but moreover have provided a clear understanding of the history of the domestication of the crop, from the ancestor wild species to the present time cultivars. The acquired knowledge of the probable diploid ancestors of most triploid cultivars allows us now to build breeding schemes that mimic the sequence of crossings and selections that have occurred over several millennia.

Recent advances in *Musa* genomics, from the first complete sequence of the *Musa* genome published in 2012 to the on-going re-sequencing of hundreds of genotypes, will doubtless increase the efficiency of banana breeding. The *Musa* genome can now be deeply explored for the characterization of desirable genes, a prelude for their use in marker-assisted selection. The very fine structure of the genome, the arrangement of the chromosomes and of the DNA sequences can now be studied with unprecedented precision, adding to the characterization of the genetic complex and the understanding of the evolutionary pathways. For banana improvement, this approach is particularly important in a biological context of gamete sterility (and difficulties to get large quantities of seeds) for which recombination and implementation of introgression strategies remain the greatest challenge.

In 2011, Heslop-Harrison wrote: "Superdomestication underpinned by genomic research with collection and assessment of the biodiversity in the genus are likely to ensure the future of the *Musa* crop" (Heslop-Harrison, 2011). That sentence is now more valid than ever before.

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Figures

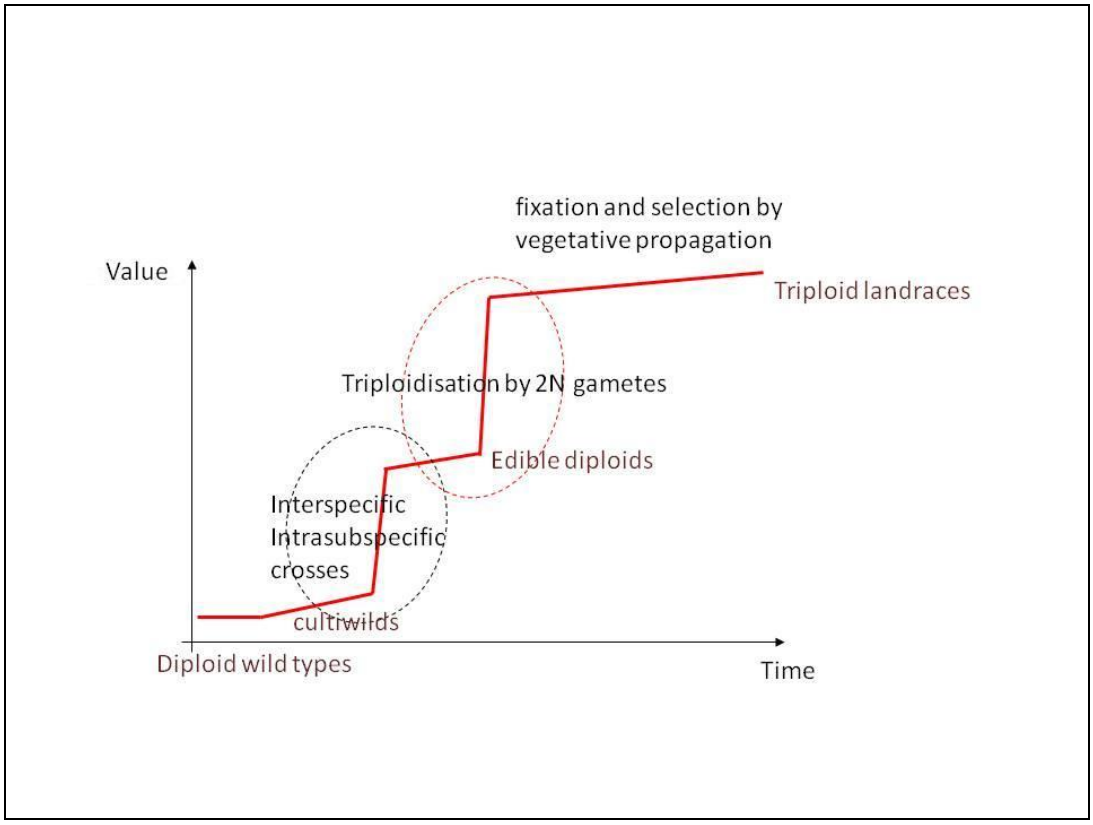

Fig. 1. Representation of the discontinuous evolution of banana by successive thresholds (From X. Perrier, pers. commun., 2013)

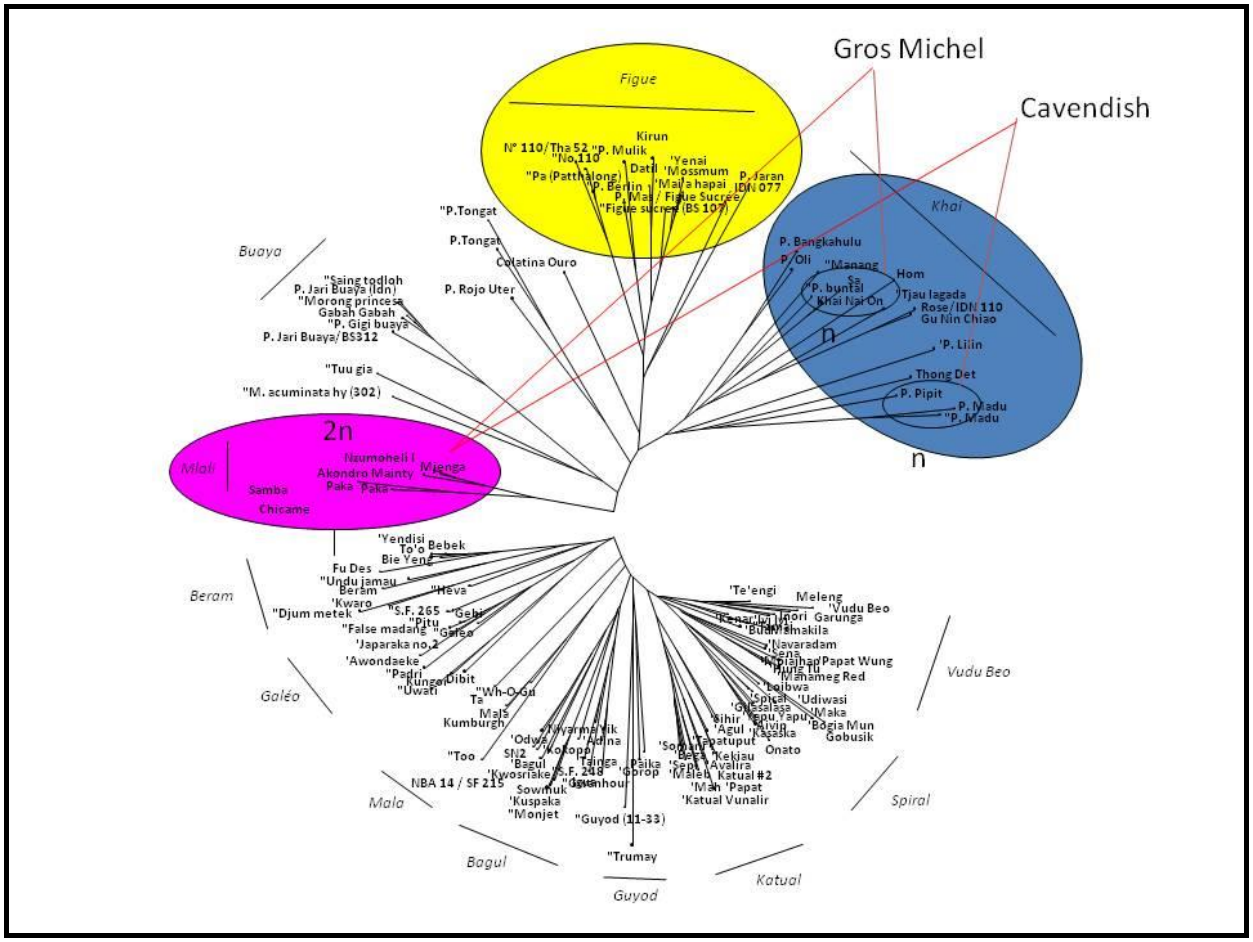

Fig. 2. Genetic structure of edible AA diploids highlighting ancestors of triploid export bananas (From: Raboin et al., 2005; Perrier et al., 2009)

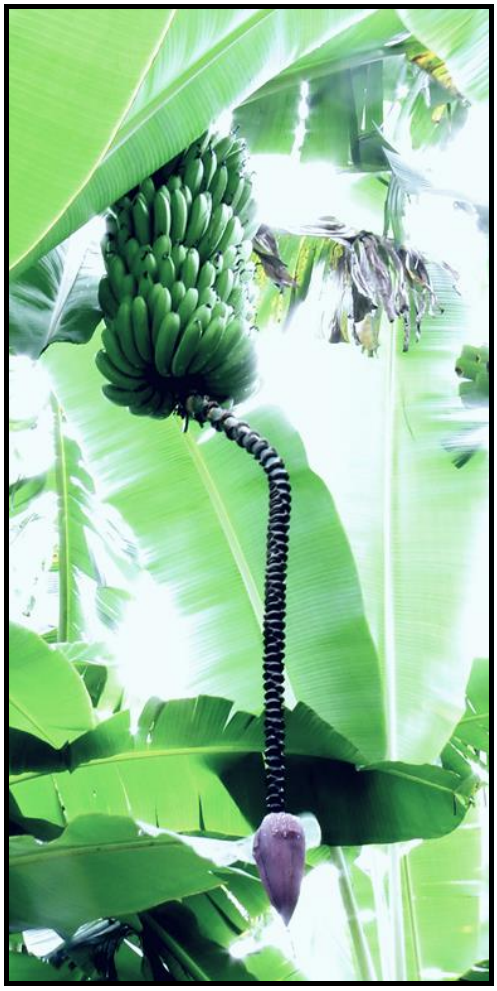

Fig. 3. “Cavendish like” AAA hybrid coming from Pisang Madu (AAcv; ♀) x “tetraploid Nzumoheli” (AAAAcv; ♂) cross.

Fig. 4. “Mysore like” AAB hybrid coming from *M. acuminata* ssp. *malaccensis* (AAw;♀) x “tetraploid Kunnan” (AABBcv; ♂) cross.